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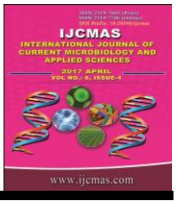

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Bacterial Profile of Vaginitis

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A B S T R A C T

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Bacterial Vaginitis is the most common vaginal infection affecting women particularly during the reproductive years. This is a complex disorder that has been associated with an increased risk of other infectious conditions of the genital tract and premature delivery in pregnancy. There is a change in vaginal ecosystem when *Lactobacilli* dominated flora is absent or greatly reduced and replaced with mixed predominately anaerobic flora. Our aim was to study the bacterial flora of women with symptoms of vaginitis and compare with the bacterial flora of control group and also to evaluate the association between alterations in the vaginal flora and clinical symptoms. The study was performed among patients with vaginal infections attending OG Department of SRM Hospital, Kattankulathur. A total of 72 samples were collected, 40 samples were from symptomatic population and 32 samples were collected from age matched women without any symptoms. However no single bacteria were shown to be the primary cause of vaginitis. This study concluded that there was no significant difference in study and control population. In the future, by using molecular techniques, we may better diagnose each subtype of BV, and able to tailor treatment appropriately.

Introduction

Reproductive tract infection (Vaginitis etc) is a common public health problem with 5 % of annual incidence and 40 million new infections occur every year. This problem has gained so much importance that it became part of Reproductive and Child Health (RCH) programme which was introduced in October 1997 (American college of Obstetricians and Gynecologists, 2011; Andersonb *et al*., 2004; Amsel *et al*., 1983).

Vaginitis can be infectious or non-infectious. Infective vaginitis may be caused by bacteria, fungi, parasites and viruses. It is said that the bacteriological agents associated with vaginitis include a wide variety of bacteria that dominate by overgrowth causing deficiency of *Lactobacilli* which play an important role to maintain the balance of vaginal flora (Anukam *et al*., 2006; Brown *et al*., 2004; Betty *et al*., 2007).

Many studies have been conducted to establish the etiological role of particular bacteria which causes bacterial vaginitis (Brown *et al*., 2004; Betty *et al*., 2007). But difficulties are encountered due to many facts; difficulty in defining Bacterial vaginitis, since in some patients it can be asymptomatic; difficulty in isolation of causative organism etc. Moreover the prevalence of bacterial vaginitis also varies with population. Study of vaginal bacterial flora among women with symptomatic vaginitis may throw some light as to the bacterial profile in relation to the type of clinical presentation (Betty *et al*., 2007; Cruickshank *et al*., 1934; Cohressen *et al*., 2005).

The main aim includes to study the bacterial flora of women with symptomatic vaginitis and to compare the bacterial flora in healthy asymptomatic women, also to evaluate associations between alterations in the vaginal flora and clinical symptoms.

Materials and Methods

The study was performed during March 2011-December 2011 among Patients with vaginal infections who attended OG Department of SRM Hospital, Kattankulathur. Symptomatic cases with white discharge and lower abdominal pain and other symptoms like pain, itching, burning in vagina were considered as the study population and Age matched normal females were taken as the control population. Women during menstruation were excluded from the study.

Samples were collected from posterior fornix or lateral vaginal wall of non-menstruating women with an Ayre's spatula using a non-lubricated speculum. Vaginal swabs and discharge fluid on slides were collected in duplicate.

Macroscopic Examination (Odour test)

Whiff test: The test was performed by adding a drop of 10% potassium hydroxide to the sample and a fishy smell indicated a positive test

Microscopic examination

Clue cells examination: The gram stained smear of the sample was examined for the presence of vaginal epithelial cells which were completely covered by gram variable coccobacilli so that, their edges which normally had a sharply defined cell border, were indistinct or stippled and considered as Clue cells.

Wet mount examination: One drop of distilled water was added to the vaginal discharge and screened for the presence of yeast cells and *Trichomonas vaginalis*.

Results and Discussion

Totally 72 samples were collected of which 40 samples represented study population from symptomatic population and 32 samples were collected from age matched women without any symptoms as control group (fig:1)

In study population, out of 51 isolates 14 were *Staphylococcus aureus* (27.45%) 13 (25.5%) were *Lactobacillus* species, followed by 10(19.6%), *Escherichia coli*, *Pseudomonas aeruginosa* 6(11.8%), *Enterococci* species 4 and 7.8%), *Klebsiella pneumonia* 3(5.9%) *and* Beta Hemolytic *Streptococci* 1(2%). In control population, out of 48 isolates 16(38%) were *Lactobacillus* spp, 11(26.2%) were *Staphylococcus aureus* followed by *Pseudomonas aeroginosa* 6(14.2%) *Escherichia coli* 5(1%), *Enterococci* spp (4.8%) and *Klebsiella pneumoniae* 2(4.8%) (Fig. 2).

In study population among 40 samples 16 women had (40%) irregular menstruation and 24 (60%) of them had regular menstruation. In control population out of 32 samples 12 women (37.5%) had irregular menstruation and 20 of them had regular menstruation (62.5%) (Fig. 3).

None of the samples from study population grew *Lactobacillus* alone, whereas only one from control population grew only *Lactobacillus* (Fig. 4). In study population, out of 13 samples which grew *Lactobacillus* 9 samples (69.2%) grew one isolate in addition to *Lactobacillus* while in control population out of 16 samples which grew *Lactobacillus* 13 (81.2%) grew one isolate in addition to *Lactobacillus*. 4/13 (31%) study population grew two isolates in addition to *Lactobacilli* while it was only 2/16 in control population which grew two isolates in addition to *Lactobacilli*.

16/40(40%) study population but only 8/32 (25%) control did not grow *Lactobacilli* from their samples. 11/6 (68.75%) in study population and 7/8 (87.5%) grew only one isolate; 5/16 (31.25%) and only 1/8 (12.5%) grew 2 isolates from study population and control population respectively (Fig. 5). On comparing the preliminary tests (Fig. 6) only 3/40 (7.5%) samples from study population was positive for whiff test while none of the 32 control samples were whiff test positive. Clue cells were seen in 8/40 (20%) of study population while 2/32 (6.2%) of control population also showed clue cells. *Trichomonas vaginalis* was seen in 3/40 (7.5%) study samples while none of the control samples were positive for *Trichomonas vaginalis.*

Two major clinical presentations were analyzed with respect to the

- Presence of clue cells
- Whiff test positivity
- *Trichomonas vaginalis* in wet mount and
- Bacterial isolates.

Abdominal pain was present in 9/40 (22.5%) study population. Among this 9 patients, 2 showed clue cells (22.2%) and GPB was present in Gram stain of 3/9(33.33%). None of them were positive for *Trichomonas vaginalis* in wet mount. Staphylococci was isolated in 2/9(22.2%) and *Lactobacilli* was isolated from 4/9(44.4%).

White discharge was the principal complaint only in 7/40(17.5%) patients. In direct smear, clue cells were positive in 2/7(28.5) and GPB in 1/7(14.2%) *Trichomanas vaginalis* was positive in 1/7(14.2%). *Lactobacilli* was isolated in 2/7(28.5%) along with 2 more isolates. Isolates other than *Lactobacilli* were seen in 5/7 (71.4%). This group of patients with white discharge showed the evidence of infection, by the presence of bacteria in the absence/reduction of *Lactobacilli* which obviously protects the vagina from gross infection. The statistical significance could not be calculated because of the small number of patients with this complaint of white discharge among the study population.

Symptomatic patients belonged to a wide range of age from 20-60 years. There was no relationship between symptoms of bacterial vaginitis and, marital status or menstrual history.

Lactobacilli are supposed to play an important role in preventing bacterial vaginitis. Hence analysis was done to compare the isolated presence of cultivable *Lactobacilli* and also *Lactobacilli* with one or more isolate other than *Lactobacilli* among the study and control group. *Lactobacilli* with two isolates were seen in more percentage of study population (30.8%) compared to control (12.5%) population. But this difference was not statistically significant (P value 0.3489)

While 16 (40%) 16/40 study population grew isolates other than *Lactobacilli* from their sample; only 8/32 (25%) of control population grew isolates other than *Lactobacilli* from their samples. This was also not significant statistically (p value 0.3195)

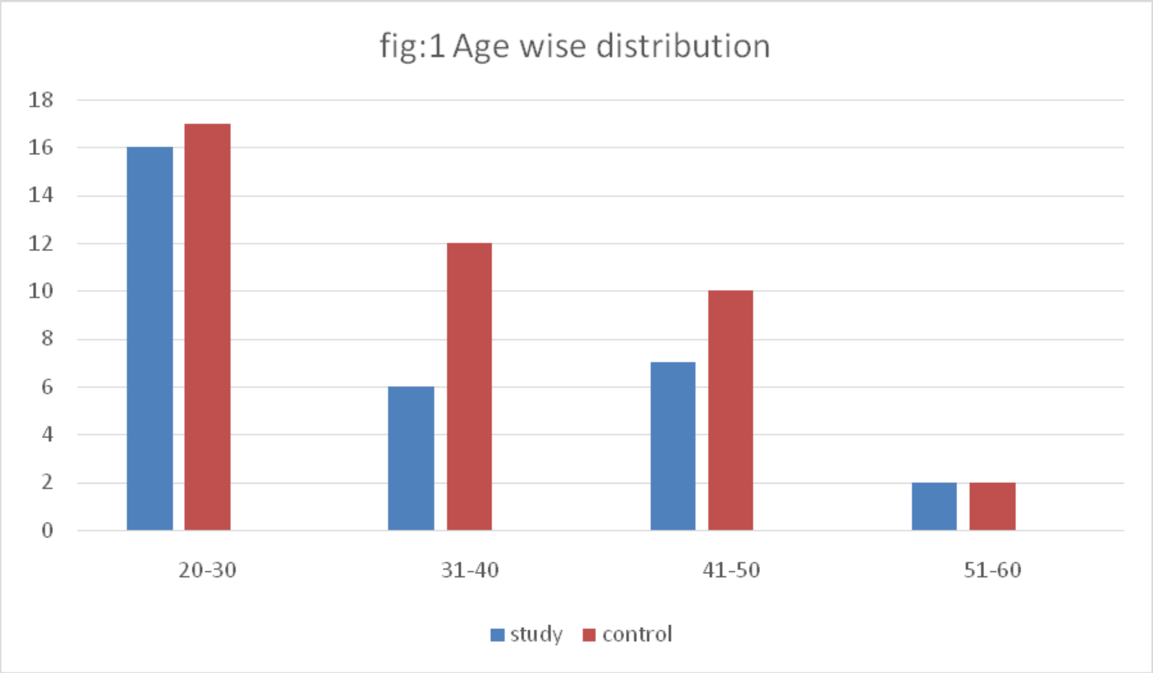
Comparison of isolates for the study and control population did not show significant difference between the two populations, though there was an apparent increase in the percentage of *Lactobacilli* isolated from control population (38%) whereas it was only 25.5% among study population. Neither this was statistically significant (p value 0.7619)

Culture is considered to be the gold standard in any infection. But bacterial vaginosis is considered to be alteration of vaginal flora without any obvious signs of inflammantion. According to Sobel and Rao there is no single best method for the diagnosis of bacterial vaginosis. Hence this attempt of studying the vaginal flora in study and control populations was undertaken. But this study also does not throw any clear cut demarcation between the asymptomatic and symptomatic group (Cohressen *et al*., 2005; Collee *et al*., 1989; Doderlein *et al*., 1892).

Lamont *et al*., observed that normal vaginal flora is not static but undergoes shifts in their representation, abundance and virulence over time and are affected by many factors. Culture and microscopy of normal vaginal flora typically shows a predominance of *Lactobacillus* species, which are believed to promote a healthy vaginal milieu.

Cultivation based techniques fail to detect fastidious organisms and thus under estimate the diversity of vaginal microbial flora.

Even though molecular techniques have identified more than 120 species of *Lactobacilli*, healthy vaginal flora did not appear to contain big numbers of many different species of *Lactobacillus* but just one or two species predominantly. Racial variation, geographical area apart from food habits which influence the GI tract and hence the vagina seemed to determine the resident *Lactobacillus* species vagina.



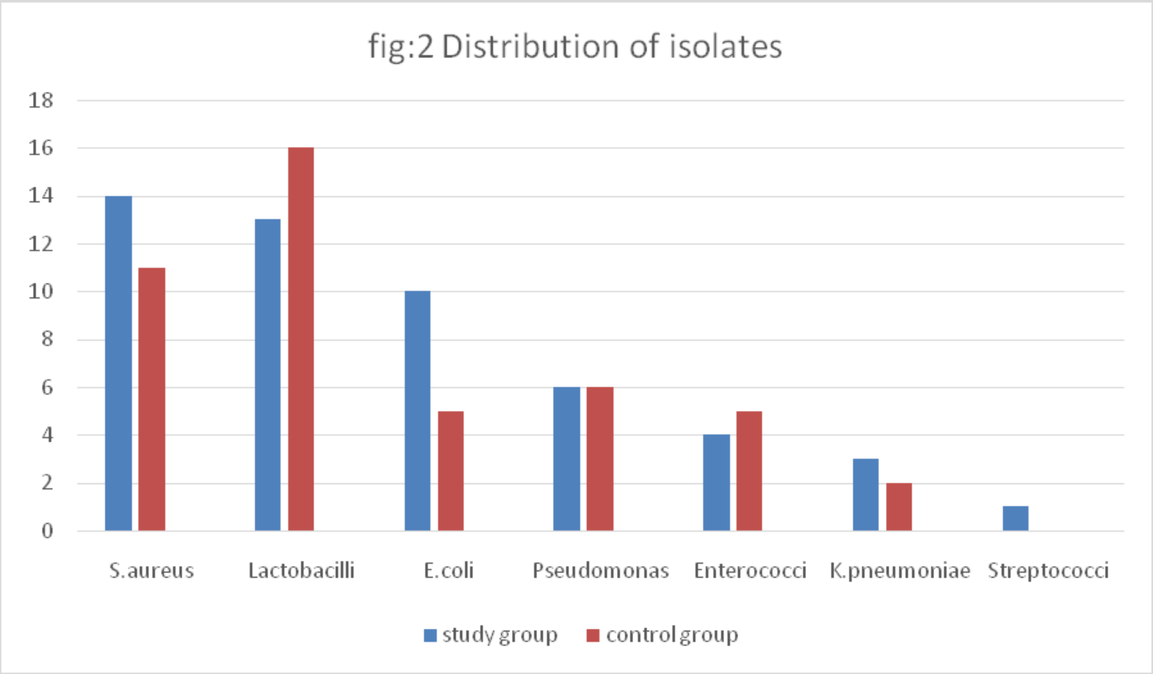


fig:2 Distribution of isolates

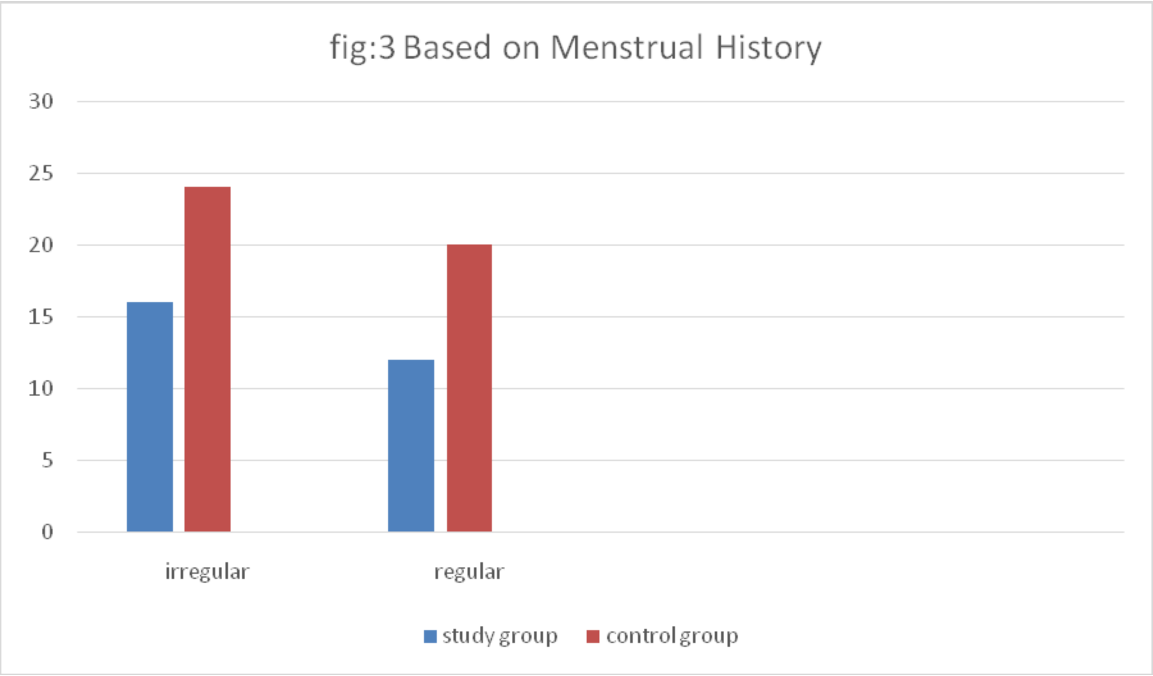


fig:3 Based on Menstrual History

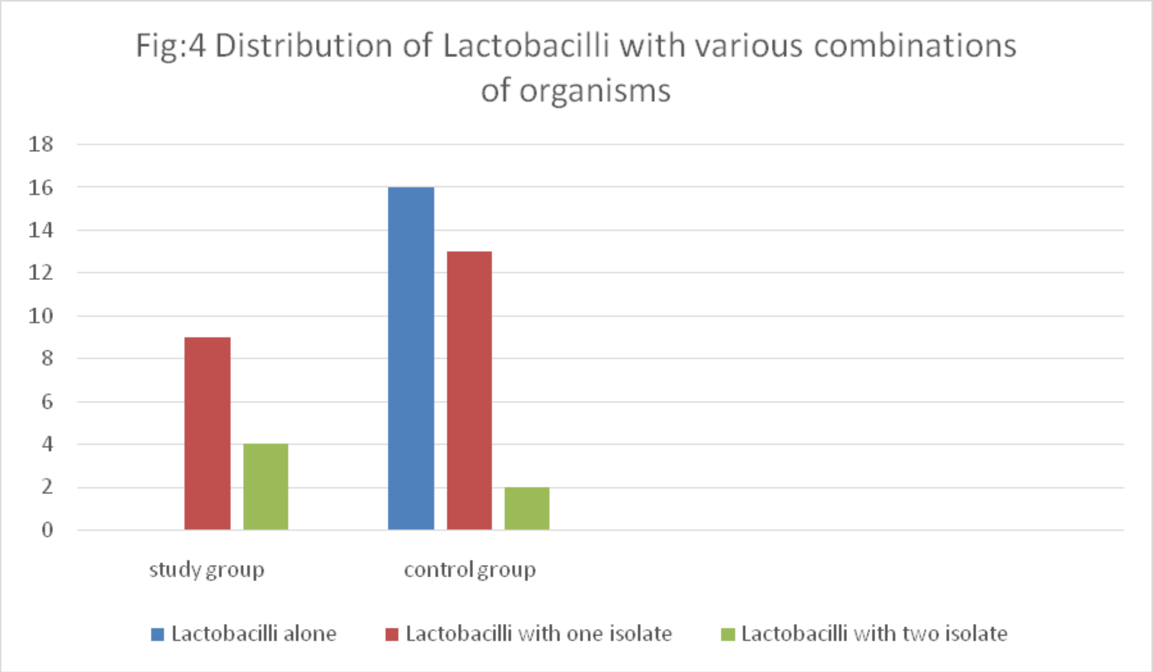


Fig:4 Distribution of Lactobacilli with various combinations of organisms
18
16
14
12
10
8
6
4
2
0
study group
control group
🟦 Lactobacilli alone
🟥 Lactobacilli with one isolate
🟩 Lactobacilli with two isolate

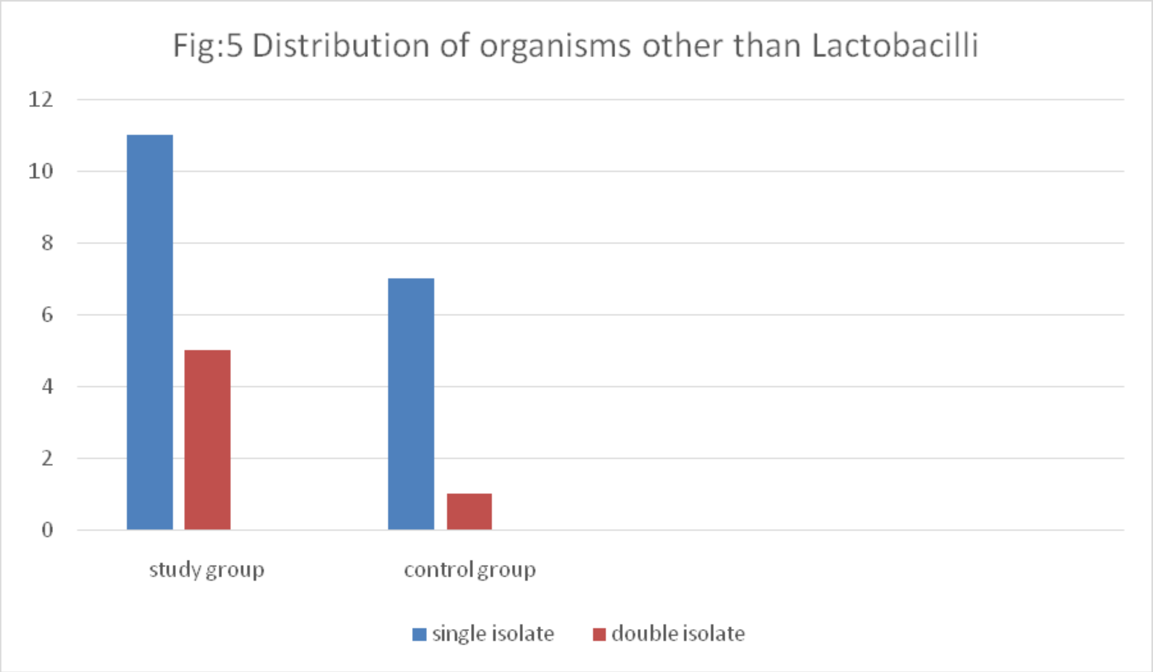
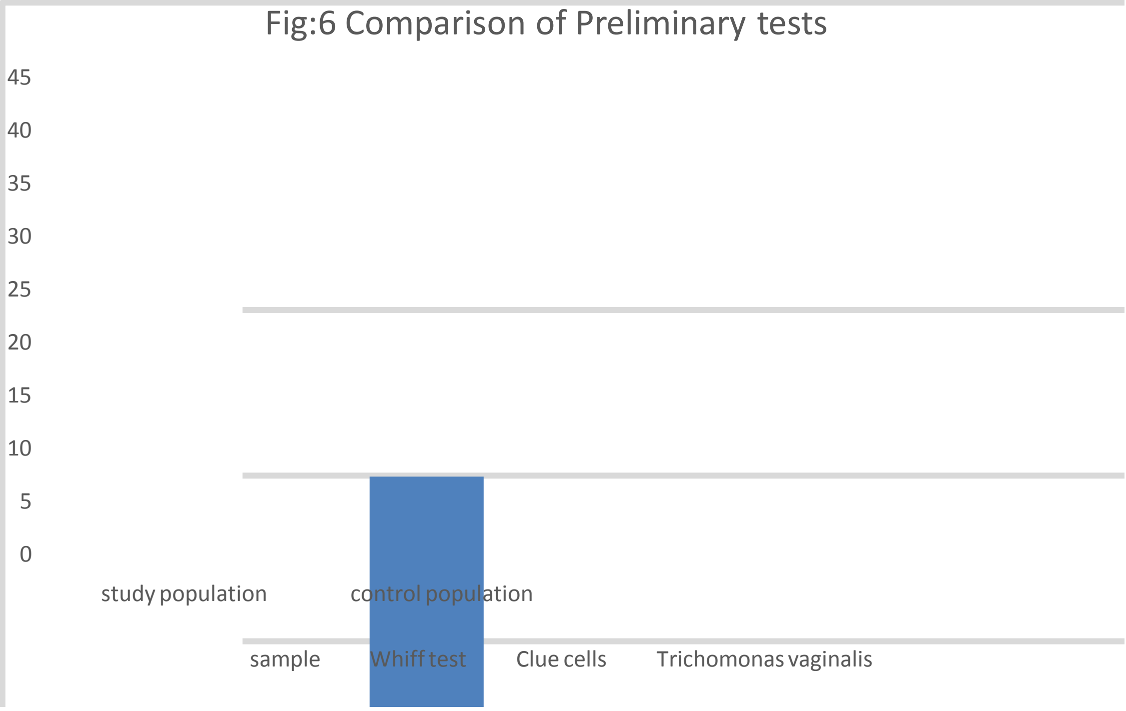


Fig:5 Distribution of organisms other than Lactobacilli
12
10
8
6
4
2
0
study group
control group
🟦 single isolate
🟥 double isolate



By using various molecular based techniques, Amesel's clinical criteria, or Nugent score to classify the normal or abnormal flora, a number of studies have demonstrated a high diversity of organisms in women with bacterial vaginosis compared to women with normal flora.

Molecular based techniques also indicate that bacterial vaginosis is not a single entity but a syndrome of variable composition that causes a variety of symptoms, and different phonotypical outcomes.

Molecular quantification in future may be able to subtype the BV and help to predict the outcome of appropriate therapy.

This study attempted to study the profile of vaginal flora among symptomatic women with that of age matched asymptomatic healthy women. Even though an apparent increase in the *Lactobacilli* was evident among asymptomatic group, the difference from symptomatic group was not statistically significant. There was no relationship between marital status and menstrual history with symptoms of bacterial vaginitis. This study also did not reveal any clear cut demarcation between the asymptomatic and symptomatic group with reference to bacteria other than *Lactobacilli*. No single bacteria were shown to be the primary cause of vaginitis.

Molecular techniques aimed to identify and quantify bacteria flora may throw a light into the exact etiology of bacterial vaginitis.

This study concluded that there was no significant difference in study and control population. In the future, by using molecular techniques, we may better diagnose each subtype of BV, and able to tailor treatment appropriately. By understanding the vaginal microbial flora we can prevent sexually transmitted diseases and especially during pregnancy, we may able to predict and prevent some of the great obstetric syndromes like PPROM, Preterm labor and Preterm birth, which are associated with infection and significant infant mortality and morbidity.

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